Phytochemistry, Vol. 28, No. 3, pp. 741–744, 1989.
Printed in Great Britain.

0031-9422/89 $3.00+0.00

LIGHT-DEPENDENT FORMATION OF GLANDULAR TRICHOMES AND MONOTERPENES IN THYME SEEDLINGS

TAKAO YAMAURA, SHIGEO TANAKA and MAMORU TABATA

Faculty of Pharmaceutical Sciences, Kyoto University, Kyoto 606, Japan

(*Received* 3 *June* 1988)

Key Word Index—*Thymus vulgaris*; Labiatae; thyme; glandular trichome; essential oil; monoterpene; thymol; γ-terpinene; *p*-cymene; light.

Abstract—The production of monoterpenes could be induced by irradiating the etiolated seedlings of thyme (*Thymus vulgaris*) with light. The yield of thymol, the main constituent of the essential oil, increased with increasing time of irradiation. The formation of peltate glandular trichomes (PGTs) occurred prior to monoterpene accumulation in both green and etiolated seedlings. GC analyses of the isolated PGTs showed that monoterpenes were accumulated mostly in the PGTs of cotyledons and primary leaves in green seedlings. Furthermore, the amount of monoterpenes in various organs of the seedlings was closely correlated to the number of PGTs. These results suggested that the primary site of monoterpene accumulation are the PGTs, the formation of which is a prerequisite for the accumulation of essential oils in this plant.

INTRODUCTION

It has been reported for labiate plants such as *Perilla frutescens* and *Pogostemon cablin* that the content of essential oils in the leaves is correlated with the number of glandular trichomes [1, 2]. This is in agreement with the demonstration of monoterpene accumulation in the isolated glandular trichomes of peppermint [3] and sage [4]. However, the biosynthesis of monoterpenes in many plants is known to be influenced by such environmental factors as temperature and nutrition [see 5 for a review].

Burbott *et al.* [6] found that a long-day treatment of peppermint caused an increase in the total amount of monoterpenes. The photoperiod is also known to affect the composition of monoterpenes as well as the growth of sweet basil [7]. Under insufficient light conditions, the monoterpene content was reduced in *Hedeoma drummondii* [8], whereas it was hardly influenced in *Satureja douglasii* in spite of a significant change in the composition of monoterpenes [9]. Thus, the effect of light on monoterpene metabolism is still unclear. Furthermore, littler is known about the role of light in the differentiation of glandular trichomes.

The present study was undertaken to investigate the effects of light on the formation of peltate glandular trichomes (PGTs) and monoterpene accumulation in thyme seedlings. A new method for isolating intact PGTs from cotyledons is also described.

RESULTS AND DISCUSSION

Effect of light on glandular trichome formation

Figure 1 shows the time course for the formation of peltate glandular trichomes (PGTs) in thyme seedlings grown at 25° in the light (2.83 W/m², 16 hr/day) or in the dark for 12 days after germination. Seeds started to root two days after germination, casting off the seed coat on day 3, irrespective of the presence of light. However, irradiation of seedlings with light promoted the development of cotyledons, while etiolated seedlings in the dark elongated their hypocotyls without expanding their cotyledons (Fig. 1A). Primary leaves were developed only in the seedlings under illumination on day 10. The number and size of PGTs in cotyledons increased sharply between day

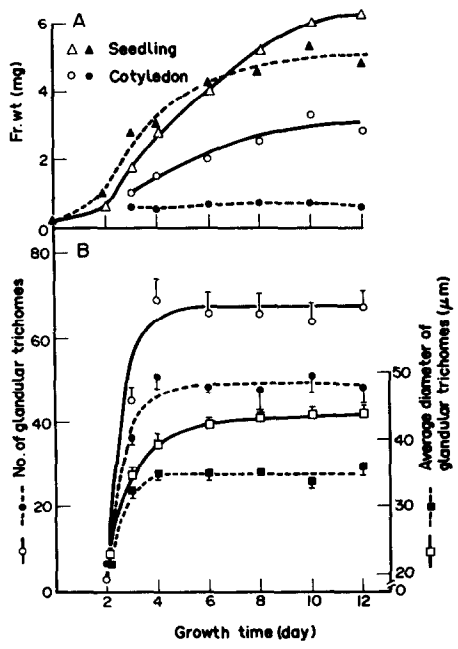

Fig. 1. Development of peltate glandular trichomes (PGTs) in the cotyledons of thyme seedlings grown under illumination (16 hr/day) with white light (solid line) or in the dark (broken line). Bars in the graph indicate standard errors.

2 and 4 both in the light and in the dark; however, the cotyledons of light-grown seedlings formed 1.3 times as many PGTs as those grown in the dark (Fig. 1B). The enlargement of PGTs was no longer observed in etiolated seedlings after day 4 when the average diameter of PGTs, was 35 μm, whereas in the light it continued at a gradually reduced rate until day 10 to reach an average diameter of 42 μm.

Effect of light on monoterpene formation

Figure 2 shows changes in the quantities of monoterpenes (γ-terpinene, *p*-cymene, thymol and carvacrol) in the cotyledons and hypocotyls of 2- to 12-day-old seedlings. In seedlings illuminated for 16 hr/day, thymol, which is biosynthesized from γ-terpinene via *p*-cymene [10], began to accumulate in cotyledons three days after germination with a cessation of new PGT formation. Its content increased at a greater rate than the other monoterpenes, reaching a maximum on day 8 (Fig. 2A). Monoterpenes, including thymol, were accumulated in hypocotyls only in small amounts (Fig. 2B), and none of them could be detected in radicles. In etiolated seedlings, on the other hand, only traces of monoterpenes were detected in cotyledons and hypocotyls after the development of PGTs. These results indicate that PGT differentiation takes place prior to monoterpene accumulation in both light- and dark-grown seedlings, and that light markedly stimulates the formation of PGTs and essential oils, especially in cotyledons.

To examine the response of cotyledons to light at different developmental stages, seedlings that had been grown in the dark for one to seven days after germination were irradiated with light for 24 hr before harvest. Four-day-old etiolated seedlings were found to be most sensitive to light, showing the greatest increase in the amount of thymol as well as in the number of PGTs (Fig. 3). Interestingly, an increase in the latter was accompanied by an increase in the thymol content on the following day.

The time of exposure of four-day-old etiolated seedlings to light sufficient for stimulating thymol production was estimated by irradiating them for various periods of time (10 min to 10 hr) before they were returned to darkness (Fig. 4). The results showed that the production of thymol was stimulated even by a 10 min-irradiation. Independently of the exposure time, there was

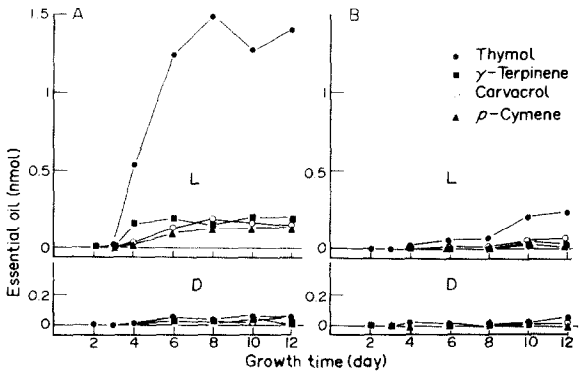

Fig. 2. Monoterpene accumulation in the cotyledons (A) and hypocotyls (B) of thyme seedlings grown under illumination (16 hr/day) (L) or in the dark (D).

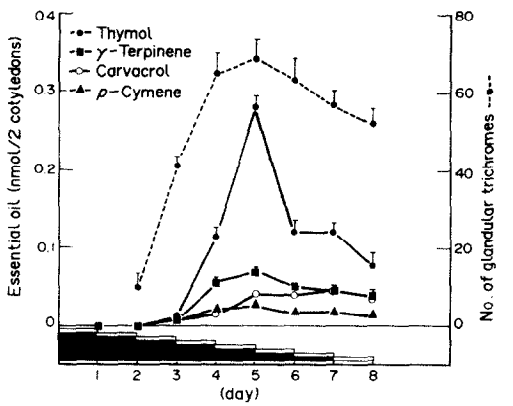

Fig. 3. Monoterpene accumulation and PGT formation in the cotyledons of thyme etiolated seedlings irradiated with white light at various growth stages.

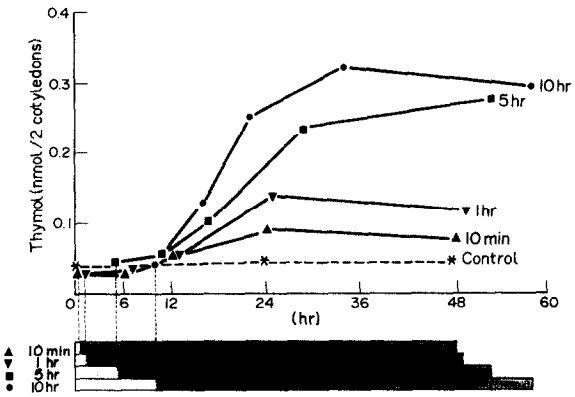

Fig. 4. Effect of irradiation for a short period of time on thymol formation in four-day-old etiolated seedlings.

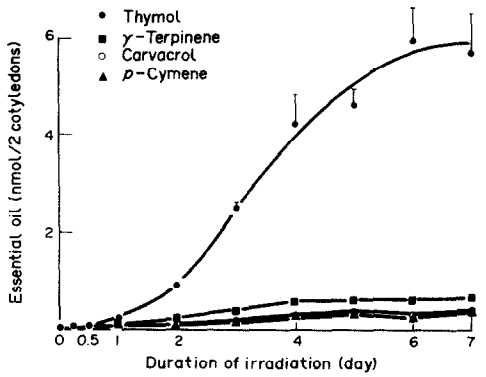

Fig. 5. Effect of continuous illumination on monoterpene formation in four-day-old etiolated seedlings.

a lag time of *ca* 10 hr before thymol began to accumulate. This lag period is presumably required for the synthesis of the aromatic monoterpene. The amount of thymol in cotyledons increased with increasing time of irradiation. Such a relationship was also observed when etiolated seedlings were exposed to light continuously for a longer period of time (Fig. 5), suggesting that the synthesis of

Table 1. Distribution of peltate glandular trichomes in various organs of 10-day-old thyme seedlings grown under illumination with white light (2.83 W/m², 16 hr/day)

Organ	Number of replicates	Number of glandular trichomes	Number of replicates	Amount of essential oils* (nmol)
A pair of cotyledons	10	70.9 ± 4.8†	3	1.85
Hypocotyl	10	5.0 ± 1.0	3	0.34
Radicle	10	0	3	0

*Total amount of γ-terpinene, *p*-cymene, thymol, and carvacrol.
†Mean ± s.e.

thymol is light-dependent. Croteau *et al.* [11] obtained a better incorporation of radioactive mevalonic acid into the monoterpenes of *Mentha* leaves by a simultaneous feeding of unlabelled sucrose, suggesting that sucrose may be satisfying the energy requirement at the biosynthetic site. However, it is not clear whether monoterpene production in thyme also depends on the supply of sucrose through photosynthesis.

Site of monoterpene accumulation

To verify that monoterpenes are accumulated in the PGTs of thyme seedlings, a large number of PGTs were isolated from either cotyledons or primary leaves of light-grown, 10-day-old seedlings by using an adhesive tape. Capillary GC analysis of an ether extract of the isolated PGTs gave a chromatogram similar to that of the whole leaf or of the cotyledon. The major components were thymol (most abundant) and γ-terpinene. Also present were small amounts of carvacrol and *p*-cymene. Microscopic examination showed that the total number of PGTs was approximately 14 times greater on the adaxial epidermis of the cotyledon than on the hypocotyl. PGTs were not found on either the abaxial epidermis of the cotyledon or the radicle epidermis (Table 1).

Although thyme possesses both peltate and capitate glandular trichomes [12], the total amount of monoterpenes in the ograns of seedlings was found to be closely related to the number of PGTs, as was reported for the leaves of *Perilla* [1] and sage [13]. Furthermore, the number of PGTs was increased by irradiation with light prior to monoterpene accumulation. These findings suggest that the developing PGT serves as a site of biosynthesis and/or accumulation of monoterpenes in this plant.

EXPERIMENTAL

Plant material. Ca 240 seeds of *Thyme vulgaris* L. were germinated in a beaker on cotton layers saturated with Shive's R–552 soln (20 ml) containing $Ca(NO_3)_2 \cdot 4H_2O$ (1230 mg/l), KH_2PO_4 (2450), $MgSO_4 \cdot 7H_2O$ (3700), $FePO_4 \cdot 4H_2O$ (7.4), H_3BO_3 (0.6), $MnCl_2 \cdot 4H_2O$ (0.4), $ZnSO_4$ (0.05), $CuSO_4 \cdot 5H_2O$ (0.05) and $H_2MoO_4 \cdot 4H_2O$ (0.02). The seedlings were incubated in a growth chamber kept at a relative humidity of 60% and a temp. of 25° under illumination with white light (2.83 W/m²) from fluorescent lamps (FL 20S · PG, National) or in the dark.

Microscopy. Seedlings were fixed in a 2% aq. soln of formaldehyde. The number and diameter of peltate glandular trichomes (PGTs) in each organ (cotyledon, hypocotyl and radicle) of 10 seedlings were counted and measured under an inverted light microscope (Diaphoto-TMD, Nikon).

Isolation of PGTs. For mechanical isolation of large numbers of PGTs, the upper surfaces of cotyledons or leaves were pressed lightly against an adhesive tape (25 mm long, 18 mm wide, Kokuyo). Then, a small amount of dist. H_2O was sprayed with a perfume spray on to the surface of the tape to detach PGTs from the sticky side of the tape. The PGTs floating on the water were sucked into a glass capillary tube (80–100 μm diameter) under microscopic observation. About 400 PGTs were collected for GC analysis of the essential oil.

Analysis of essential oils. One hundred organs excised from seedlings were extracted with Et_2O (0.2 ml) containing *p-tert*-butylphenol as an internal standard. Usually, each treatment consisted of 6 replicates. The quantities of monoterpenes contained in the extracts were analysed by GC (Hitachi 163) under the following conditions: column: 10% PEG 20M on chromosorb W (3 m × 3 mm, glass column), carrier gas: N_2 20 ml/min, column temp.: 40–180° (4°/min), injection temperature: 230°, detection: FID.

Et_2O extracts of the isolated PGTs were analysed by means of capillary GC: column: Ulbon HR-54 (25 m × 0.24 mm), carrier gas: N_2 1.2 ml/min, column temp.: 130°, injection temp.: 180°, detection: FID. Identification of monoterpenes (γ-terpinene, *p*-cymene, thymol, and carvacrol) was carried out by a GCMS (JEOL JMS-DX300/JMA-DA-5000 system) comparison with authentic samples.

REFERENCES

1. Yoshida, T., Higashi, F. and Ikawa, S. (1968) *Proc. Crop Sci. Soc. Jpn* **37**, 118.
2. Henderson, W., Hart, J. W., How, P. and Judge, J. (1970) *Phytochemistry* **9**, 1219.
3. Amelunxen, F., Wahlig, T. and Arbeiter, H. (1969) *Z. Pflanzenphysiol.* **61**, 68.
4. Venkatachalam, K. V., Kjonaas, R. and Croteau, R. (1984) *Plant Physiol.* **76**, 148.
5. Bernáth, J. (1986) in *Herbs, Spices, and Medicinal Plants: Recent Advances in Botany, Horticulture, and Pharmacology*, Vol. 1. (Craker, L. E. and Simon, J. E., ed.), p. 185. Oryx Press, Phoenix.
6. Burbott, A. J. and Loomis, W. D. (1967) *Plant Physiol.* **42**, 20.

7. Skrubis, B. and Markakis, P. (1976) *Econ. Botany* **30**, 389.
8. Firmage, D. H. (1981) *Biochem. Syst. Ecol.* **9**, 53.
9. Lincoln, D. E. and Langenheim, J. H. (1978) *Biochem. Syst. Ecol.* **6**, 21.
10. Poulose, A. J. and Croteau, R. (1978) *Arch. Biochem. Biophys.* **187**, 307.
11. Croteau, R., Burbott, A. J. and Loomis, W. D. (1972) *Phytochemistry* **11**, 2937.
12. Werker, E., Ravid, U. and Putievsky, E. (1985) *Israel J. Botany* **34**, 31.
13. Croteau, R., Felton, M., Karp, F. and Kjonaas, R. (1981) *Plant Physiol.* **67**, 820.